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**Published***With international search report.**Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.***(54) Title:** PROCESS FOR MAKING COMPLIANT DEHYDRATED TISSUE FOR IMPLANTATION**(57) Abstract**

A process for preparing pliable soft tissue specimen which are resistant to cracking and devoid of viable cells includes the steps of treating native soft tissue obtained from a donor by a gradually increasing gradient of aliphatic alcohol or other suitable water miscible polar organic solvent until the last alcohol (or other solvent) solution has at least 25 % by volume of the organic liquid. Thereafter, the tissue specimen is treated with a solution containing glycerol or low molecular weight (< 1000D) polyethylene glycol, and polyethylene glycol of a molecular weight between approximately 6,000 to 15,000 D and heparin. Thereafter, the tissue specimen is briefly immersed in aqueous heparin solution, frozen and lyophilized. The tissue specimen is suitable for implantation as a homograft or xenograft, with or without rehydration.

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## 1           PROCESS FOR MAKING COMPLIANT DEHYDRATED TISSUE FOR IMPLANTATION

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## 4           BACKGROUND OF THE INVENTION

## 5        1. Field of the Invention

6           The present invention is in the field of implant materials. More  
7           particularly, the present invention is directed to compliant dehydrated implant  
8           materials which have no viable cells, and can be stored and transported  
9           without being immersed in liquid. The present invention is also directed to  
10          the process of producing said implant materials.

## 11       2. Brief Description of the Prior Art

12          The use of autografts, homografts and xenografts for augmenting or  
13          replacing defective tissues in humans and animals has been known for a long  
14          time. From the standpoint of providing suitable materials for implantation,  
15          augmenting or replacing hard tissues, such as bone, presents a different type of  
16          problem than augmenting or replacing soft tissues. In the selection of  
17          substitute materials for hard tissue graft, the strength and hardness of the graft  
18          are important whereas compliance and flexibility are, generally speaking, less  
19          crucial.

20          On the other hand, in the selection of soft tissue materials for  
21          implantation, compliance and flexibility of the graft material are usually of  
22          utmost importance because the soft tissue replacement material usually must  
23          closely match the healthy functional tissue that will be replaced. In this  
24          regard it must be remembered that natural soft tissue containing collagen is  
25          strong and able to withstand repeated three-dimensional stress as well as  
26          bending and deformation. Often natural soft tissue acts as a physical barrier  
27          that must maintain its structural integrity. Ideally, replacement or  
28          augmentation soft tissue that is utilized in implantation should have the same  
29          characteristics as the natural soft tissue that it replaces, and should be easy to  
30          obtain, store and transport. These, however are difficult goals that the prior

1 art has been striving to attain, and up to the present invention only with  
2 moderate success.

3 More particularly, in accordance with one major approach in the prior  
4 art to preserve soft tissue for eventual implantation, tissues of human or animal  
5 origins have been treated with chemical modifiers/preservatives, such as  
6 glutaraldehyde, which cross-links collagen and other proteins. The  
7 glutaraldehyde treated tissues have been shown to be adequately resistant to  
8 mechanical fatigue as well as biodegradation when implanted in human  
9 patients. However, the glutaraldehyde cross-linking alters the viscoelastic  
10 properties of tissues, and therefore, as a result of host response undesirable  
11 calcification and build-up of peripheral granulation tissues usually occur in the  
12 implants with time. Glutaraldehyde is an effective biocidal (sterilizing) agent,  
13 but when exposed to air it slowly loses its biocidal effectiveness. Therefore,  
14 the tissue intended for implantation (bioprosthetic) must be kept in  
15 glutaraldehyde solution during storage and transportation and the package  
16 including the glutaraldehyde soaked bioprosthetic must be kept tightly sealed.  
17 Moreover, it must not be exposed to significantly elevated temperature.  
18 Because of these requirements the costs of utilizing glutaraldehyde-treated  
19 soft tissue bioprostheses are high. Glutaraldehyde is toxic, and therefore it  
20 must be carefully removed from the bioprosthetic by rinsing before  
21 implantation. This represents still another disadvantage of glutaraldehyde-  
22 treated bioprostheses.

23 Another major approach for providing soft tissue bioprosthetic in the  
24 prior art utilizes liquid sterilants other than glutaraldehyde. Some of these  
25 alternative approaches also avoid the calcification problems associated with  
26 glutaraldehyde treated implants. However, in accordance with these  
27 processes also, to avoid brittleness and to more-or-less maintain the physical  
28 integrity of the bioprostheses the tissues have to be maintained, stored and

1 transported in liquid media up to the time immediately preceding implantation.

2 Still another alternative method for providing soft tissue bioprostheses  
3 is the use of cryo-preserved fresh tissues of homograft (tissue from the same  
4 species). Because of recent advances in cryo-preservation, the cryo-preserved  
5 fresh tissues have recently made homograft implants relatively more  
6 successful and more accepted as an alternative to glutaraldehyde-preserved  
7 xenograft. A serious disadvantage of cryo-preserved bioprostheses is the  
8 difficulty to assure that they are free of infectious disease agents. The costs of  
9 preparing and handling of cryo-preserved bioprosthetic tissues is also very  
10 high because of the need for keeping the tissues at all times below the usual or  
11 normal freezer temperatures.

12 From among the numerous patent disclosures in the prior art directed to  
13 preparing and/or preserving biological tissue for implantation or other use as  
14 replacement tissue, United States Patent Nos. 5,116,552 (*Morita et al.*) and  
15 5,336,616 (*Livesey et al.*) are mentioned as of interest to the present invention.

16 United States Patent No. 5,116,552 (*Morita et al.*) describes a process for  
17 preparing lyophilized collagen sponge for medical applications, such as  
18 artificial skin. The process of this reference comprises the steps of  
19 impregnating cross-linked collagen sponge with an aqueous solution of a  
20 hydrophilic organic solvent, freezing the sponge and thereafter vacuum drying  
21 (lyophilizing) it. However, the resulting freeze-dried product is not pliable  
22 and is not protected from cracking because the water and the hydrophilic  
23 solvent or solvents have been removed in the lyophilization step. United  
24 States Patent No. 5,336,616 (*Livesey et al.*) describes treatment of soft tissue  
25 obtained from a source, such as a cadaver, with solutions containing  
26 antioxidants, protease inhibitors and antibiotics (stabilizing solution), with  
27 enzymes and detergents to remove viable antigenic cells (processing solution),  
28 and after decellularization with a cryopreservative solution that prevents

1 destructive ice crystal formation while the tissue is frozen. The cryo-  
2 preserving solution may include a combination of organic solvent and water.  
3 After lyophilization the product is stored and transported in a sealed container  
4 in an inert gas atmosphere, thus protected from atmospheric moisture. Prior to  
5 implantation the tissue is rehydrated and must be restored with  
6 immunotolerable viable cells to produce a permanently acceptable graft for  
7 implantation.

8 Still other disclosures pertaining to the preparation and/or preservation  
9 of biological tissue for implantation, or related subjects, can be found in  
10 United States Patent Nos. 2,106,261; 2,610,625; 2,645,618; 3,939,260;  
11 4,277,238; 4,280,954; 4,300,243; 4,383,832; 4,578,067; 4,703,108; 4,704,131;  
12 4,760,131; 4,801,299; 4,911,915; 5,028,597; 5,131,850; 5,674,290 and U.K.  
13 Patent Specification 716,161.

#### 14 SUMMARY OF THE INVENTION

15 It is an object of the present invention to provide a soft tissue graft  
16 suitable for implantation in humans or other mammals which graft after  
17 rehydration has substantially the same mechanical properties as the natural soft  
18 tissue from which the graft was obtained.

19 It is another object of the present invention to provide a soft tissue graft  
20 that satisfies the foregoing objective, that is also devoid of viable cells and  
21 does not require inoculation with viable cells prior to implantation.

22 It is still another object of the present invention to provide a soft tissue  
23 graft that satisfies the foregoing objectives, that can be stored and transported  
24 in a dehydrated form.

25 The foregoing and other objects and advantages are attained by a soft  
26 tissue preparation that in its dehydrated state is compliant, resists cracking, is  
27 devoid of viable cells and which is obtained by successively treating natural  
28 soft tissue:

1           with liquid compositions of gradually increasing concentrations of a C<sub>1</sub>  
2   - C<sub>3</sub> alcohol, or other polar water miscible organic solvent in water, until the  
3   last of said liquid compositions contains at least approximately 25 % by  
4   volume alcohol, or the other organic solvent, or mixtures thereof, the balance  
5   being water;

6           thereafter with a second liquid composition of aqueous glycerol or of  
7   low molecular weight (<1000D) polyethylene glycol, containing the glycerol  
8   or the low molecular weight polyethylene glycol, or mixtures thereof, in a  
9   concentration range of approximately 10 to 50 % by volume, said second  
10   liquid composition also containing approximately 3 - 20 % weight by volume  
11   polyethylene glycol of a molecular weight in the range of 6,000 D to 15,000 D  
12   and approximately 2 to 75 unit per milliliter heparin of a molecular weight  
13   greater than approximately 3KD;

14           thereafter draining excess liquid from the soft tissue so treated;  
15           thereafter immersing the soft tissue in an aqueous heparin solution of  
16   approximately 20 to 500 unit per milliliter concentration, and  
17           thereafter freezing the tissue and lyophilizing the tissue to dryness.

18           The features of the present invention can be best understood together  
19   with further objects and advantages by reference to the following detailed  
20   description of specific examples and embodiments.

## 21           DESCRIPTION OF THE PREFERRED EMBODIMENTS

22           The following specification sets forth the preferred embodiments of the  
23   present invention. The embodiments of the invention disclosed herein are the  
24   best modes contemplated by the inventors for carrying out their invention,  
25   although it should be understood that various modifications can be  
26   accomplished within the parameters of the present invention.

27           In accordance with the present invention soft tissue intended for graft in  
28   mammals, including humans, is first obtained from a source, such as cadavers.

1      Bovine, ovine, porcine tissue and soft tissue obtained from other animals, such  
2      as sheep, serve as examples. Human soft tissue may also be used.  
3      Homografts, that is tissues implanted in the same species as the donor, as well  
4      as xenografts, that is tissues implanted in species different from the donor, can  
5      be prepared in accordance with the present invention. The types of tissues  
6      used in accordance with the present invention are generally the same which are  
7      normally used in state-of-the-art surgical procedures involving implantations  
8      of soft tissues, primarily in humans. Examples of tissues frequently utilized in  
9      these procedures are pericardium, aortic and pulmonary roots, tendons,  
10     ligaments, skin, peritonium, pleura, mitral and tricuspid valves.

11       The soft tissue excised from the donor is usually trimmed to remove  
12      loose excess or unneeded tissue and fat. Usually the tissue is then kept in  
13      saline solution. Thereafter, and in accordance with the present invention, the  
14      tissue is treated in a first aqueous solution containing a C<sub>1</sub> - C<sub>3</sub> alcohol in  
15      relatively low concentration (approximately 15 - 35), and thereafter in a  
16      second aqueous solution of greater alcohol concentration, in the range of  
17      approximately 25 to 75 % volume by volume. (All concentrations described  
18      in this application are volume by volume, unless specifically stated otherwise.)

19       The purpose of the treatment of the tissue specimen with the first and second  
20      solutions is to gradually replace the water content of the specimen with  
21      alcohol. Methyl, ethyl and iso-propyl alcohols can be used for this purpose  
22      with ethyl alcohol being preferred. Other, non-toxic polar and water miscible  
23      organic solvents e.g. acetonitrile, acetone or methyl-ethyl ketone can also be  
24      used instead of the above-listed alcohols, and mixtures of alcohols and organic  
25      solvents are also suitable for use in the invention. Preferably, the first  
26      solution contains approximately 25 % ethyl alcohol, the balance being water,  
27      and the second solution contains approximately 50 % ethyl alcohol, the  
28      balance being water.

1        Those skilled in the art will readily recognize that the foregoing  
2        manipulations represent treatment of the tissue specimen with a stepwise  
3        increasing gradient of alcohol (or other suitable non-toxic water miscible  
4        organic solvent) concentration, until a concentration of at least approximately  
5        25 %, preferably approximately 50 %, and at most approximately 75 %  
6        alcohol (or other suitable solvent) concentration is reached. Instead of  
7        treating the tissue specimen with the aforesaid concentration gradient in two  
8        steps, the specimen could also be treated with the gradient in three or more  
9        steps, or even with a continuously increasing gradient until the upper limit of  
10      the alcohol (or other suitable solvent) concentration is reached. The treatment  
11      with the increasing gradient of alcohol (or other suitable solvent) concentration  
12      is conducted at ambient temperature and is best performed by immersing the  
13      tissue specimen in the solutions. The timing of the exposure of the tissue  
14      specimen to these solutions is not critical and is somewhat dependent on the  
15      thickness of the specimen. However sufficient time must be given for the  
16      solution to penetrate the specimen. Typically, 30 minutes are sufficient and in  
17      the preferred embodiments of the process of the invention the tissue specimen  
18      are kept for approximately 30 minutes in each of the first and second alcohol  
19      solutions.

20       After immersion (treatment) in the above-described alcohol solutions,  
21      the tissue specimen is immersed (treated) in a third solution that contains  
22      approximately 10 to 50 % glycerol, approximately 3 to 20 % weight by  
23      volume polyethylene glycol of a molecular weight in the range of 6,000 D to  
24      15,000 D and approximately 2 to 75 unit per milliliter heparin of a molecular  
25      weight greater than approximately 3KD. Preferably, the third solution  
26      contains approximately 20 % glycerol, approximately 5 % (weight by volume)  
27      polyethylene glycol that has a molecular weight of approximately 8,000 D and  
28      approximately 50 unit per milliliter heparin. Instead of glycerol, a low

1 molecular weight (<1000 D) polyethylene glycol can be included in the third  
2 solution. The duration of immersion in the third solution is also not critical,  
3 approximately 30 minutes are sufficient for very thin tissues such as ovine,  
4 porcine, bovine or human pericardium, but for thicker tissues longer times of  
5 exposure, such as 6 hours, or preferably 12 hours are convenient and preferred.

6 After treatment with the third solution, the tissue specimen is removed  
7 therefrom and excess liquid is allowed to drain from the specimen. The  
8 specimen is then briefly (for seconds as in a quick dip) immersed in, or is  
9 otherwise treated with aqueous heparin solution of approximately 20 to 500  
10 unit/ml concentration, and preferably of approximately 250 ml/unit  
11 concentration, then the heparin solution is allowed to drain off. Thereafter, the  
12 specimen is frozen in a manner usual in the art for freezing specimens prior to  
13 lyophilization. Those skilled in the art understand that freezing is usually  
14 conducted in a freezer of ultra-low temperature, that is between approximately  
15 - 60°C - -80°C. After freezing, the tissue specimen is lyophilized (dried *in*  
16 *vacuo*) in a manner known in the art.

17 Tissue samples processed in accordance with the invention tend to be  
18 translucent and have a slight yellowish tint in color. Unlike tissues lyophilized  
19 from 100% water or physiological saline solution, the tissues of the invention  
20 are pliable, compliant and do not crack or break as a result of physical  
21 manipulations.

22 For use in surgical procedures as an implant, and for most tests  
23 conducted in accordance with the present invention to compare the treated  
24 tissues with fresh tissues, the lyophilized tissues are first rehydrated in  
25 physiological buffered saline. This is done by treating, preferably by  
26 immersing, the lyophilized tissue of the invention in physiological buffered  
27 saline solution for approximately 5 minutes to one hour. The rehydrated  
28 tissues of the invention have an appearance that is practically

1 indistinguishable from the appearance of the fresh tissue. Rehydration is  
2 typically conducted at ambient temperature. It can be done, other than in  
3 saline, in the patient's own blood, in tissue culture medium, and in low  
4 percentage (<10%) ethyl alcohol solution. A preferred method of rehydrating  
5 tissue specimen in accordance with the present invention is in buffered saline  
6 of pH 7.4.

7 As noted above, except for testing the tissue specimen of the present  
8 invention, rehydration is performed only prior to use of the tissue specimen  
9 for implantation. Otherwise the specimen are stored and transported at  
10 ambient temperature in a sealed container protected from atmospheric  
11 moisture. The lyophilized tissues can be readily sterilized by gas phase  
12 sterilization methods, and can also be implanted without first being rehydrated.  
13

14 The tissue specimen of the invention do not contain viable cells, but  
15 tests described below demonstrated that after rehydration the tissue specimen  
16 are not cytotoxic and are compatible for host endothelial cells to attach and  
17 proliferate on them. This attachment and proliferation of host cells and lack  
18 of cytotoxicity are important for long term survival of most implants. The  
19 tissues of the invention are hemocompatible and resistant to platelet  
20 aggregation and thrombus formation. Tests, described below, also  
21 demonstrated that the collagen fibers of the native tissue have remained  
22 substantially intact during the steps of the process of the invention, and are  
23 substantially intact in the rehydrated tissue.

24 Specific Examples and Description of Tests

25 (a) preparation of lyophilized bovine or ovine pericardium

26 Fresh bovine and ovine pericardium was cut into strips and squares  
27 were dissected to remove loose tissues and fat. The tissues were immediately  
28 placed in aqueous 25% ethyl alcohol solution for 30 minutes. The aqueous

1        25% ethyl alcohol solution was replaced by aqueous 50% ethyl alcohol  
2        solution for another 30 minutes. The second (50% ethyl alcohol) solution  
3        was then replaced for approximately 16 hours by a third solution containing  
4        20% glycerol, 5% weight by volume polyethylene glycol (MW 8,000) and 50  
5        unit/ml heparin (molecular weight >3KD). The tissues were carefully  
6        removed from the third solution, excess liquid was allowed to drain from the  
7        tissues and the tissues were dipped in a heparin solution of 250 unit/ml for a  
8        few seconds, prior to freezing the tissues at -70 ° C. The completely frozen  
9        tissues were lyophilized to dryness.

10              The lyophilized bovine or ovine pericardium tissues obtained above had  
11        a translucent appearance and a slight yellowish tint. They were pliable and  
12        did not crack or break by physical manipulations. They could be rehydrated  
13        by immersion in physiological buffered saline for approximately 5 minutes at  
14        ambient temperature. After rehydration, the tissues were indistinguishable in  
15        appearance from the native fresh tissues.

16              Human fibroblasts and umbilical cord vein endothelial cells were  
17        cultured on the rehydrated pericardium tissues to study their biocompatibility.  
18        Round discs of the tissues were cut to fit the bottom of the wells of a 24 well  
19        culture plate. Plastic rings were placed on top of the tissues to hold the tissues  
20        down and to ensure a good seal at the edge of the tissues. Cells were seeded  
21        on the tissues in normal culture media for one week. At the end of the  
22        incubation period, tissues were recovered and cut into different portions for  
23        histology studies. Histological examination of the cross-section of the tissues  
24        showed a thin layer of endothelial cells adhering to the surface of the tissues.  
25        Cells on the tissues were also released by trypsin and counted. These results  
26        showed that the rehydrated tissues are not cytotoxic and are biocompatible for  
27        host cells to attach and proliferate. As is known, attachment and proliferation  
28        of endothelial cells and other connective tissue cells on cardiac implants is

1     essential for the long term survival of the implant.

2                 The integrity of the collagen fibers in the treated tissues was examined  
3     by melting temperature measurements. For these, tissues were heated in  
4     phosphate buffered saline from 37 ° C until they shrunk. The shrinkage  
5     temperature of the fresh native tissues and of the lyophilized and rehydrated  
6     tissues in accordance with the present invention was approximately the same,  
7     at approximately 63+1 ° C, indicating that the collagen fibers remained intact  
8     throughout the lyophilization and rehydration process.

9     (b) preparation of lyophilized sheep aortic and pulmonary roots

10               Aortic and pulmonary roots of donor sheep were also treated with the  
11     aqueous 25% ethyl alcohol, aqueous 50% ethyl alcohol, aqueous 20%  
12     glycerol 5% polyethylene glycol, and subsequent heparin solution and  
13     lyophilized, as described above for the bovine and ovine pericardium.

14               The treated roots were rehydrated and implanted as homografts in the  
15     descending aorta of host sheep. Our results show that after 100 days of  
16     implantation, the valves were competent and the roots do not appear different  
17     from the un-implanted native tissues. The hundred-day explant was free of  
18     fibrin deposition and free of host tissue reaction. The leaflets of the valve  
19     appeared intact and indistinguishable from the unimplanted valve by both  
20     gross observation and histological examination.

## 1    WHAT IS CLAIMED IS:

- 2    1. A process for preparing a pliable soft tissue specimen, comprising  
3       the steps of:  
4           (1) treating natural soft tissue obtained from a donor with:  
5                   (a) liquid compositions of gradually increasing concentrations of  
6                      a polar organic solvent or solvents, until the last of said liquid  
7                      compositions contains at least approximately 25 % by volume of said  
8                      solvent, or mixture of solvents, the balance being water, the solvent  
9                      being selected from a group consisting of aliphatic alcohols having 1 to  
10                  3 carbons and other water miscible polar organic solvents;  
11                   (b) thereafter with a second liquid composition of aqueous  
12                  glycerol or of low molecular weight polyethylene glycol having a  
13                  molecular weight less than approximately 1000D, the glycerol or the  
14                  low molecular weight polyethylene glycol, or mixtures thereof being in  
15                  a concentration range of approximately 10 to 50 % by volume, said  
16                  second liquid composition also containing approximately 3 - 20 %  
17                  weight by volume of polyethylene glycol of a molecular weight in the  
18                  range of 6,000 D to 15,000 D and approximately 2 to 75 unit per  
19                  milliliter heparin of a molecular weight greater than approximately  
20                  3KD;  
21                   (2) thereafter briefly immersing the soft tissue in an aqueous heparin  
22                  solution, and  
23                   (3) thereafter freezing the tissue and lyophilizing the tissue to dryness.  
24    2. The process in accordance with Claim 1 wherein the polar  
25       organic solvents are selected from the group consisting of methyl alcohol,  
26       ethyl alcohol, iso-propyl alcohol, acetonitrile, acetone and methyl ethyl  
27       ketone.  
28    3. The process in accordance with Claim 2 wherein the polar

1        organic solvent is ethyl alcohol.

2            4.      The process in accordance with Claim 1 wherein the natural soft  
3        tissue obtained from the donor is treated with liquid compositions of gradually  
4        increasing concentrations of a polar organic solvent or solvents, until the last  
5        of said liquid compositions contains at least approximately 50 % by volume  
6        of said solvent, or mixture of solvents.

7            5.      The process in accordance with Claim 4 where the polar organic  
8        solvent is ethyl alcohol.

9            6.      The process in accordance with Claim 1 wherein the second  
10      liquid composition contains approximately 20 % by volume of glycerol.

11           7.      The process in accordance with Claim 1 wherein the natural soft  
12      tissue is treated in succession with two liquid compositions of a polar organic  
13      solvent or solvents, the first of said compositions containing approximately 15  
14      to 35 % by volume of the solvent or solvents, the second of said composition  
15      containing approximately 25 to 75 % by volume of the solvent or solvents.

16           8.      The process in accordance with Claim 7 wherein the polar  
17      organic solvent is ethyl alcohol.

18           9.      The process in accordance with Claim 1 further comprising the  
19      step of rehydrating the lyophilized tissue specimen.

20           10.     A process for preparing a pliable soft mammalian tissue  
21      specimen, for eventual implantation in a mammal to replace or augment native  
22      tissue, the process comprising the steps of:

23           (1)     treating natural soft mammalian tissue obtained from a donor  
24      with:

25                (a)    liquid compositions of gradually increasing  
26        concentrations of an aliphatic alcohol or mixture of aliphatic alcohols  
27        having 1 to 3 carbon atoms, until the last of said liquid compositions  
28        contains at least approximately 25 % by volume of said alcohol or

1 mixture of alcohols, the balance being water;

2 (b) thereafter with a second liquid composition of aqueous  
3 glycerol containing the glycerol in a concentration range of  
4 approximately 10 to 50 % by volume, said second liquid composition  
5 also containing approximately 3 - 20 % weight by volume  
6 polyethylene glycol of a molecular weight in the range of 6,000 D to  
7 15,000 D and approximately 2 to 75 unit per milliliter heparin of a  
8 molecular weight greater than approximately 3KD;

9 (2) thereafter briefly immersing the soft tissue in an aqueous heparin  
10 solution of approximately 20 to 500 unit per milliliter concentration, and

11 (3) thereafter freezing the tissue and lyophilizing the tissue to dryness.

12 11. The process in accordance with Claim 10 wherein the aliphatic  
13 alcohol is ethyl alcohol.

14 12. The process in accordance with Claim 11 wherein the natural  
15 soft tissue is treated with said compositions containing ethyl alcohol, until the  
16 last of said compositions contains at least approximately 50 % by volume ethyl  
17 alcohol.

18 13. The process in accordance with Claim 12 wherein the  
19 concentration of glycerol in the second liquid composition is approximately 20  
20 % by volume.

21 14. The process in accordance with Claim 13 wherein the  
22 concentration of polyethylene glycol in the second liquid composition is  
23 approximately 5 % weight by volume and the molecular weight of said  
24 polyethylene glycol is approximately 8,000 D.

25 15. The process in accordance with Claim 14 wherein the natural  
26 soft tissue is treated in succession with two liquid compositions of ethyl  
27 alcohol, the first of said compositions containing approximately 15 to 35 % by  
28 volume of ethyl alcohol, the second of said composition containing

1 approximately 25 to 75 % by volume of ethyl alcohol.

2       16. The process in accordance with Claim 16 further comprising the  
3 step of rehydrating the lyophilized tissue specimen.

4       17. The process in accordance with Claim 10 wherein the natural soft  
5 mammalian tissue is from pericardium, pleura, peritonium from aortic,  
6 pulmonary mitral or tricuspid valves, or from tendon or skin.

7       18. A pliable soft tissue specimen which has been prepared in a  
8 process comprising the steps of:

9           (1) treating natural soft tissue obtained from a donor with:

10              (a) liquid compositions of gradually increasing concentrations of  
11                 a polar organic solvent or solvents, until the last of said liquid  
12                 compositions contains at least approximately 25 % by volume of said  
13                 solvent, or mixture of solvents, the balance being water, the solvent  
14                 being selected from a group consisting of aliphatic alcohols having 1 to  
15                 3 carbons and other water miscible polar organic solvents;

16              (b) thereafter with a second liquid composition of aqueous  
17                 glycerol or of low molecular weight polyethylene glycol having a  
18                 molecular weight less than approximately 1000D, containing the  
19                 glycerol or the low molecular weight polyethylene glycol, or mixtures  
20                 thereof being in a concentration range of approximately 10 to 50 % by  
21                 volume, said second liquid composition also containing approximately  
22                 3 - 20 % weight by volume polyethylene glycol of a molecular weight  
23                 in the range of 6,000 D to 15,000 D and approximately 2 to 75 unit per  
24                 milliliter heparin of a molecular weight greater than approximately  
25                 3KD;

26              (2) thereafter briefly immersing the soft tissue in an aqueous heparin  
27                 solution, and

28              (3) thereafter freezing the tissue and lyophilizing the tissue to dryness.

1           **19.** The pliable soft tissue specimen in accordance with Claim 18  
2       wherein in the process of preparing the specimen the polar organic solvents are  
3       selected from the group consisting of methyl alcohol, ethyl alcohol, iso-propyl  
4       alcohol, acetonitrile, acetone and methyl ethyl ketone.

5           **20.** The pliable soft tissue specimen in accordance with Claim 18  
6       wherein in the process of preparing the specimen the natural soft tissue  
7       obtained from the donor is treated with liquid compositions of gradually  
8       increasing concentrations of a polar organic solvent or solvents, until the last  
9       of said liquid compositions contains at least approximately 50 % by volume  
10      of said solvent, or mixture of solvents.

11          **21.** The pliable soft tissue specimen in accordance with Claim 20  
12       wherein in the process of preparing the specimen the polar organic solvent is  
13       ethyl alcohol.

14          **22.** The pliable soft tissue specimen in accordance with Claim 18  
15       wherein in the process of preparing the specimen the second liquid  
16       composition contains approximately 18 % by volume of glycerol.

17          **23.** The pliable soft tissue specimen in accordance with Claim 18  
18       wherein in the process of preparing the specimen the natural soft tissue is  
19       treated in succession with two liquid compositions of a polar organic solvent  
20       or solvents, the first of said compositions containing approximately 15 to 35 %  
21       by volume of the solvent or solvents, the second of said composition  
22       containing approximately 25 to 75 % by volume of the solvent or solvents.

23          **24.** The pliable soft tissue specimen in accordance with Claim 18  
24       wherein the process of preparing the specimen further comprises the step of  
25       rehydrating the lyophilized tissue specimen.

26          **25.** A pliable soft tissue specimen, for eventual implantation in a  
27       mammal to replace or augment native tissue, which has been prepared in a  
28       process comprising the steps of:

- 1           (1)     treating natural soft mammalian tissue obtained from a donor  
2                 with:
  - 3                 (a)    liquid compositions of gradually increasing  
4                 concentrations of an aliphatic alcohol or mixture of aliphatic alcohols  
5                 having 1 to 3 carbon atoms, until the last of said liquid compositions  
6                 contains at least approximately 25 % by volume of said alcohol or  
7                 mixture of alcohols, the balance being water;
  - 8                 (b)    thereafter with a second liquid composition of aqueous  
9                 glycerol containing the glycerol in a concentration range of  
10                 approximately 10 to 50 % by volume, said second liquid composition  
11                 also containing approximately 3 - 20 % weight by volume polyethylene  
12                 glycol of a molecular weight in the range of 6,000 D to 15,000 D and  
13                 approximately 2 to 75 unit per milliliter heparin of a molecular weight  
14                 greater than approximately 3KD;
- 15           (2) thereafter briefly immersing the soft tissue in an aqueous heparin  
16             solution of approximately 20 to 500 unit per milliliter concentration, and  
17           (3) thereafter freezing the tissue and lyophilizing the tissue to dryness.
- 18           26.     The soft tissue specimen in accordance with Claim 25 wherein in  
19             the process of preparing the specimen the aliphatic alcohol is ethyl alcohol.
- 20           27.     The soft tissue specimen in accordance with Claim 26 wherein in  
21             the process of preparing the specimen the natural soft tissue is treated with  
22             said compositions containing ethyl alcohol, until the last of said compositions  
23             contains at least approximately 50 % by volume ethyl alcohol.
- 24           28.     The soft tissue specimen in accordance with Claim 27 wherein in  
25             the process of preparing the specimen the concentration of glycerol in the  
26             second liquid composition is approximately 20 % by volume.
- 27           29.     The soft tissue specimen in accordance with Claim 28 wherein in  
28             the process of preparing the specimen the concentration of polyethylene

1       glycol in the second liquid composition is approximately 5 % weight by  
2       volume and the molecular weight of said polyethylene glycol is approximately  
3       8,000 D.

4           **30.**   The soft tissue specimen in accordance with Claim 29 wherein in  
5       the process of preparing the specimen the natural soft tissue is treated in  
6       succession with two liquid compositions of ethyl alcohol, the first of said  
7       compositions containing approximately 15 to 35 % by volume of ethyl  
8       alcohol, the second of said composition containing approximately 25 to 75 %  
9       by volume of ethyl alcohol.

10          **31.**   The soft tissue specimen in accordance with Claim 25 wherein the  
11       process of preparing the specimen further comprises the step of rehydrating  
12       the lyophilized tissue specimen.

13          **32.**   The soft tissue specimen in accordance with Claim 25 wherein the  
14       natural soft mammalian tissue is from pericardium, pleura, peritonium, from  
15       aortic, pulmonary, mitral or tricuspid valves or from tendon and skin.

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 99/14247

**A. CLASSIFICATION OF SUBJECT MATTER**  
IPC 6 A61L27/00

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 98 07452 A (SULZER VASCUTEK LIMITED ;WALKER DONALD FRANCIS (GB)) 26 February 1998 (1998-02-26) examples 1,2,5,6 ---	1-32
A	US 5 558 875 A (WANG SU) 24 September 1996 (1996-09-24)  column 4, line 36 - line 47 --- -/-	1-5, 7, 10-12, 15, 17-21, 23, 25-27, 30, 32



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

\* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search

14 October 1999

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## INTERNATIONAL SEARCH REPORT

International Application No  
PCT/US 99/14247

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 4 357 274 A (WERNER HEINZ-HELMUT) 2 November 1982 (1982-11-02)  the whole document -----	1,6,10, 13,14, 17,18, 22,25, 28,29,32

**INTERNATIONAL SEARCH REPORT**

Information on patent family members

International Application No

PCT/US 99/14247

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9807452 A	26-02-1998	AU 3950297 A	06-03-1998
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